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Lubsch, Alexander; Timmermans, Klaas R.

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UPTAKE KINETICS AND STORAGE CAPACITY OF DISSOLVED INORGANIC PHOSPHORUS AND CORRESPONDING DISSOLVED INORGANIC NITRATE UPTAKE IN *SACCHARINA LATISSIMA* AND *LAMINARIA DIGITATA* (PHAEOPHYCEAE)¹

Alexander Lubsch² 

NIOZ Royal Netherlands Institute for Sea Research, Department of Estuarine and Delta Systems, Utrecht University, PO Box 140, 4401 NT Yerseke, the Netherlands

Department Ocean Ecosystems, University of Groningen, PO Box 72, 9700 AB Groningen, the Netherlands

and Klaas R. Timmermans

NIOZ Royal Netherlands Institute for Sea Research, Department of Estuarine and Delta Systems, Utrecht University, PO Box 140, 4401 NT Yerseke, the Netherlands

Uptake rates of dissolved inorganic phosphorus and dissolved inorganic nitrogen under unsaturated and saturated conditions were studied in young sporophytes of the seaweeds *Saccharina latissima* and *Laminaria digitata* (Phaeophyceae) using a “pulse-and-chase” assay under fully controlled laboratory conditions. In a subsequent second “pulse-and-chase” assay, internal storage capacity (ISC) was calculated based on V_M and the parameter for photosynthetic efficiency F_v/F_m . Sporophytes of *S. latissima* showed a V_S of $0.80 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ and a V_M of $0.30 \pm 0.09 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ for dissolved inorganic phosphate (DIP), whereas V_S for DIN was $11.26 \pm 0.56 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ and V_M was $3.94 \pm 0.67 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$. In *L. digitata*, uptake kinetics for DIP and DIN were substantially lower: V_S for DIP did not exceed $0.38 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ while V_M for DIP was $0.22 \pm 0.01 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$. V_S for DIN was $3.92 \pm 0.08 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ and the V_M for DIN was $1.81 \pm 0.38 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$. Accordingly, *S. latissima* exhibited a larger ISC for DIP ($27 \mu\text{mol} \cdot \text{cm}^{-2}$) than *L. digitata* ($10 \mu\text{mol} \cdot \text{cm}^{-2}$), and was able to maintain high growth rates for a longer period under limiting DIP conditions. Our standardized data add to the physiological understanding of *S. latissima* and *L. digitata*, thus helping to identify potential locations for their cultivation. This could further contribute to the development and modification of applications in a bio-based economy, for example, in evaluating the potential for bioremediation in integrated multitrophic aquacultures that produce biomass simultaneously for use in the food, feed, and energy industries.

Key index words: *Laminaria digitata*; nitrate uptake; phosphate uptake; *Saccharina latissima*; uptake kinetics

Abbreviations: DIN, Dissolved inorganic nitrate; DIP, Dissolved inorganic phosphate; DW, Dry weight; F_v/F_m , F_v refers to variable fluorescence; F_m refers to maximum fluorescence; FW, Fresh weight; IMTA, Integrated multitrophic aquaculture; ISC, Internal storage capacity; NIOZ, Royal Netherlands Institute for Sea Research; PAM (fluorometry), Pulse-amplitude-modulation (fluorometry); SA, Surface area; T, Nutrient concentration; V_e , Externally controlled uptake; V_M , Maintenance uptake rate; V_S , Surge uptake rate; V, Uptake rate

Dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) are essential macronutrients for maintaining the metabolism and growth of seaweeds. Phosphorus (P) and Nitrogen (N) are key components of nucleic acids, phospholipids, adenosine triphosphate (ATP) and are also involved in controlling enzyme reactions and in the regulation of metabolic pathways. After N, P is the second most frequently limiting macronutrient in seaweed growth. Nutrient limitation and shifts in limitation from one element to another can significantly affect the internal composition, physiology, and growth of seaweeds (Pederson and Borum 1996, Gevaert et al. 2001). These processes can reflect natural fluctuations, but can also be driven by anthropogenic emissions. For example, agricultural run-off waters contain considerable amounts of inorganic phosphate (PO_4^{3-}) and nitrogenous compounds, like nitrate (NO_3^-) and ammonium (NH_4^+ ; Sharpley et al. 1992, Rabalais et al. 2009). Anthropogenic discharge can also generate nutrient concentration gradients, which are often observed along coastal zones due to the proximity of nutrient sources. This can not only lead to alterations in the

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²Author for correspondence: e-mail alex.lubsch@gmail.com
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type and magnitude of nutrient limitations, but may also cause effects of eutrophication. In the North Sea, measures against eutrophication were first installed in the mid 1980s, when its dramatic effects on marine flora and fauna became evident (Westernhagen and Dethlefsen 1983, Malta and Verschuure 1997, Lyngby et al. 1999). Recently it showed, that the de-eutrophication efforts have led to a large imbalance in the N:P stoichiometry of coastal waters of the North Sea in north-western Europe (Burson et al. 2016). Increasing N:P ratios, which outpace the Redfield ratio of 16:1 were observed (Radach and Pätsch 2007, Grizzetti et al. 2012) and a pronounced P-limitation can be effective in coastal regions of the southern North Sea.

This can have notable effects on the ecosystem communities and growth and functioning of primary producers. It has been reported that N availability mediates the ability of primary producers to access P, as shown for the brown seaweed *Fucus vesiculosus* Linnaeus (Perini and Bracken 2014).

The perennial brown seaweeds (Phaeophyceae) *Saccharina latissima* and *Laminaria digitata* are commonly found on the lower shores of the north Atlantic around the northern North American and European coastlines, including the North Sea. *Saccharina latissima* is also distributed along the shores of the north Pacific. As ecosystem engineers, *S. latissima* and *L. digitata* can affect sedimentation and erosion by reducing water currents (Jones et al. 1994, Bouma et al. 2005) and offer shelter, feedstock, and nursery habitats to various fauna, thus enhancing the diversity of their habitat (Jørgensen and Christie 2003). Both seaweeds are rich sources of nutrients and contain large amounts of carbohydrates in the form of structural, storage, and functional polysaccharides, as well as considerable amounts of proteins (Holdt and Kraan 2011). Aside from the direct use of *S. latissima* and *L. digitata* for culinary and medicinal purposes, there is great interest in the refinement, extraction, and application of carbohydrates and proteins in the energy and animal feed industries, as well as the extraction of important food hydrocolloids, including carrageenan and alginates (McHugh 2003, Troell et al. 2006, Holdt and Kraan 2011). However, the content of these compounds varies, depending on nutrient availability, temperature, light, and hydrodynamics, alternating in accordance to season and area of cultivation (Murata and Nakazoe 2001, Connan et al. 2004).

The vast range of possibilities for using seaweed, especially *Saccharina latissima* and *Laminaria digitata*, has resulted in an enormous surge in interest over the last decades (McLachlan 1985), hence stimulating the efforts toward large-scale cultivation as a supplement to wild harvests (Neori 2008, Bixler and Porse 2011, Holdt and Kraan 2011, Kraan 2013). Although there is much known about the growth requirements of *S. latissima* (Bartsch et al. 2008, Reid et al. 2013, Marinho et al. 2015) and *L. digitata* (Bolton and Lüning 1982, Schaffelke and Lüning 1994,

Harrison and Hurd 2001, Gordillo et al. 2002, Pederson et al. 2010), there is relatively little information available about the DIP uptake kinetics, as well as DIP and DIN management in relation to the internal storage capacity (ISC), the maximal internal duration for growth under external limiting conditions (Pederson et al. 2010). This important information, as it allows an estimation of ecological effects on nutrient availability and can contribute to development and modification of cultivation sites. A lot of studies related to uptake kinetics for DIN and DIP in *Saccharina latissima* and *Laminaria digitata* have been conducted under field conditions with weekly to monthly sampling intervals (Bolton and Lüning 1982, Schaffelke and Lüning 1994, Reid et al. 2013, Marinho et al. 2015) and the majority of studies under laboratory conditions have focused on uptake of nitrogenous compounds, as NO_3^- and NH_4^+ , in *S. latissima* and *L. digitata* (Chapman et al. 1978, Conolly and Drew 1985, Harrison et al. 1986). Often DIN and DIP uptake is tested independently in short term experiments, usually ranging from minutes to hours (e.g., Runcie et al. 2003, Martínez and Rico 2004, Luo et al. 2012). Long-term responses to DIN and DIP availability remain unknown.

Nutrient uptake by seaweed can be split into three distinct phases, referred to as surge uptake (V_S), metabolic or internally controlled uptake (V_M), and externally controlled uptake (V_E ; Conway et al. 1976, Harrison et al. 1989). V_S refers to the filling of internal nutrient pools, uncoupled from growth (Conway et al. 1976), and has often been described for nutrient-starved seaweeds (e.g., Fujita 1985, Harrison et al. 1989, Dy and Yap 2001). The uptake rates gradually decrease as internal nutrient pools in cytoplasm and vacuoles are filled (Rosenberg et al. 1984, Fujita 1985). When internal nutrient concentrations are constant and relative uptake rates of nutrients remain relatively stable over time, V_M , which is considered equal to the rate of assimilation, is attained (Taylor and Rees 1999, Barr et al. 2004). The previously filled nutrient pools can be utilized at times of low external nutrient availability (Probyn and Chapman 1982, Pederson and Borum 1996). The ISC and temporal duration of the filled nutrient pools under external nutrient depletion conditions has hardly been focused on in seaweeds (Fujita 1985).

Experimental studies under controlled conditions are critical to further understand the role of nutrients and shifts in nutrient ratios, and will strengthen the understanding of nutrient demand and strategies by seaweeds. This is of great ecological and economic importance, as it will open up opportunities to forecast the impacts of nutrient limitation and will shed light on possible competitive advantages of one species versus the other under shifts in limitation from one element to another. It will also facilitate to identify potential locations for seaweed mariculture and provide insight into optimal cultivation practices in regard to nutrient additions.

MATERIALS AND METHODS

In this study, we present the DIP- and DIN-uptake kinetics of young *Saccharina latissima* and *Laminaria digitata* sporophytes exposed to a range of nominal DIP concentrations ($0\text{--}6\ \mu\text{mol} \cdot \text{L}^{-1}$) and nonlimiting DIN concentration ($50\ \mu\text{mol} \cdot \text{L}^{-1}$) under laboratory conditions, controlling for temperature, light, and hydrodynamics in a “pulse-and-chase” assay (i.e., adding a pulse of nutrients and following their removal from the water over time).

In a second “pulse-and-chase” experiment under the same laboratory conditions for light, temperature, and hydrodynamics, sporophytes of both species were exposed to DIP-depleted, DIN-depleted, DIP and DIN-depleted, and DIP- and DIN-enriched seawater. Thereafter, the fluorescence signal F_v/F_m , which is a measure of plant stress/photosynthetic efficiency, was measured over 9 weeks. Based on this data, the DIP- and DIN-uptake kinetics as well as the ISC of DIP and DIN in *Saccharina latissima* and *Laminaria digitata* were quantified and standardized for surface area (SA).

All experiments and analyses were conducted at the Royal Netherlands Institute for Sea Research (NIOZ) located on Texel, the Netherlands. Cultured sporophytes of *Saccharina latissima* and *Laminaria digitata*, offspring from plants originated and collected from the coastline of Den Helder, the Netherlands, were transferred from incubation tanks at the NIOZ Seaweed Centre (www.nioz.nl/en/expertise/seaweed-research-centre) into four separate (2 for each species) transparent 20 L Nalgene™ bottles (Nalge Nunc International Corporation, Rochester, NY, USA), filled with 15 L seawater medium, inside a temperature-controlled room ($12.0 \pm 0.6^\circ\text{C}$, measured hourly by HOBO temperature loggers; Onset, Bourne, MA, USA), for an adaptation phase under fully controlled laboratory conditions. Two tubular fluorescent lamps (OSRAM L18 Watt 965, Deluxe cool daylight), attached 50 cm above the flasks and covered by two layers of black mosquito netting, provided a PAR light intensity of $18 \pm 3\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ($n = 9$; light meter ULM-500, Walz, Germany) inside the glass flasks in a set light:dark period of 16:8 h. The low light concentration was installed to avoid light induced stress on the sporophytes, as previous cultivation of young individuals in light concentrations of $70\text{--}80\ \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (after Lüning 1979, Andersen et al. 2011) in a light/dark period of 16:8 h led to frond-bleaching within 2 d (not depicted). A moderate water movement inside the 20 L bottles was provided by aeration, produced by a common air pump (AquaForte V-20; Hailea Group Co., Ltd., China, Guangdong) outside the bottles, which was connected to a PVC hose, connected to a glass pipette (25 mL) inside a bottle.

We used two experimental approaches: (i) analyzing DIP and DIN uptake kinetics under unsaturated (V_S) and saturated states (V_M) of the two seaweeds, and (ii) estimation of their ISC for DIP and DIN, based on V_M and the fluorescence protocol of F_v/F_m , as an indicator for (nutritional) stress. Based on the different two experimental approaches to determine DIP and DIN uptake kinetics and ISC for DIP and DIN, the nutrient concentration in the seawater medium inside the 20 L bottles differed during adaptation phase, whereas other parameters like light, temperature, and hydrodynamics were kept constant.

Experimental approach 1. Sporophytes of both species were maintained in nutrient-depleted seawater ($\text{PO}_4^{3-} = 0.008\ \mu\text{mol} \cdot \text{L}^{-1}$, $\text{NH}_4^+ = 0.022\ \mu\text{mol} \cdot \text{L}^{-1}$ and $\text{NO}_3^- = 0.003\ \mu\text{mol} \cdot \text{L}^{-1}$) for a 15 d adaptation phase in experimental approach 1, which is similar to the experimental set-up used for determining uptake kinetics in *Ulva lactuca* by Lubsch and Timmermans (2018). Exposing the sporophytes to nutrient-depleted seawater ensured nutrient starvation, as data for their nutritional history was not available. After this starvation phase, 49 randomly

picked sporophytes of *Saccharina latissima* and *Laminaria digitata* with a frond size range of $1.5\text{--}6.5\ \text{cm}^2$, respectively, $5.5\text{--}29.9\ \text{cm}^2$ (Fig. 1) were individually transferred into 200 mL glass jars filled with 100 mL of seawater medium enriched with a range of dissolved inorganic phosphate levels (DIP: $0.0\ \mu\text{mol} \cdot \text{L}^{-1}$, $0.2\ \mu\text{mol} \cdot \text{L}^{-1}$, $0.4\ \mu\text{mol} \cdot \text{L}^{-1}$, $0.8\ \mu\text{mol} \cdot \text{L}^{-1}$, $1.5\ \mu\text{mol} \cdot \text{L}^{-1}$, $3.0\ \mu\text{mol} \cdot \text{L}^{-1}$ and $6.0\ \mu\text{mol} \cdot \text{L}^{-1}$) and a nonlimiting concentration of DIN ($50\ \mu\text{mol} \cdot \text{L}^{-1}$). The installed DIP concentrations, as well as DIN concentration, covered the range of observed natural concentrations in coastal areas of the NE Atlantic, respectively, neighboring seas like the North Sea, which seasonal extremes (winter concentrations) show an overall average of $2\text{--}4\ \mu\text{mol} \cdot \text{L}^{-1}$ for DIP and $60\text{--}90\ \mu\text{mol} \cdot \text{L}^{-1}$ for DIN for the years 2006–2014 (OSPAR assessment report 2017; <https://www.oap.ospar.org/en/ospar-assessment/intermediate-assessment-2017/pressures-human-activities/eutrophication/nutrient-concentration>; retrieved in August 2018). The investigation on higher DIP concentrations, as in nominal concentration of $6.0\ \mu\text{mol} \cdot \text{L}^{-1}$ could be of interest for nursery operations of the seaweeds, as well as integrated multitrophic aquaculture (IMTA) activities with young sporophytes or bioremediation purposes.

The seawater medium was refreshed (“pulsed”) and samples of the day-old medium were taken (“chased”) for dissolved nutrient analysis on a daily basis for 3 weeks, as the removal of DIP and DIN from the seawater medium were referred to uptake rates by the seaweed.

After daily refreshment of the seawater medium, all flasks were randomly distributed to minimize differences in light availability on a rotating table, which provided moderate water movement at a speed of 100 rpm. This constant water movement was maintained for optimal mixing and, hence, availability of nutrients by decreasing the diffusion boundary layers between tissues and the growing medium (e.g., Gonen et al. 1995, Hurd 2000).

Seawater medium. The base for the seawater medium was nutrient-poor seawater from the North Atlantic Ocean

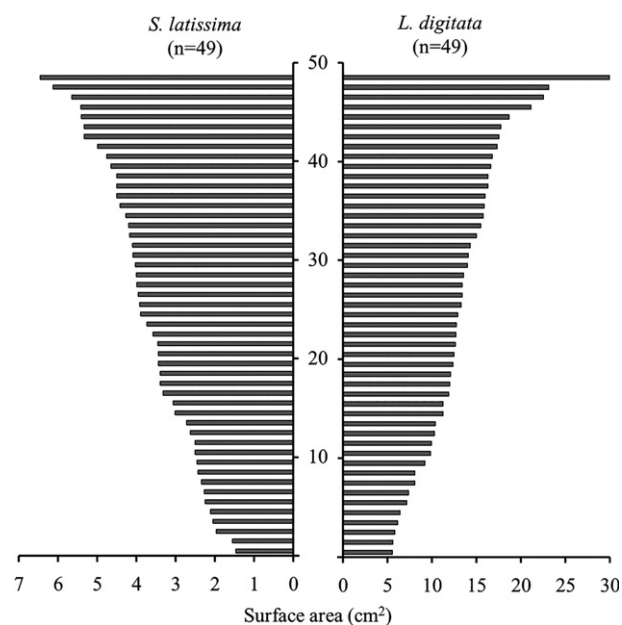


FIG. 1. Size range of initial surface area (cm^2) of *Saccharina latissima* and *Laminaria digitata* sporophytes applied in experiments on nutrient uptake kinetics (experimental approach 1).

(salinity 34.5) with low phosphate (PO_4^{3-} ; $0.008 \mu\text{mol} \cdot \text{L}^{-1}$), ammonium (NH_4^+ ; $0.022 \mu\text{mol} \cdot \text{L}^{-1}$), and nitrate (NO_3^- ; $0.003 \mu\text{mol} \cdot \text{L}^{-1}$) concentrations. The seawater was pasteurized (80°C for 2 h) and salinity was adjusted to 29.5 to reflect the values measured at the NIOZ Seaweed Research Centre and around the island of Texel by mixing with ultrapure water (Milli-Q, Merck KGaA, MA, USA). Afterward, potassium-dihydrogen-phosphate (KH_2PO_4) and potassium nitrate (KNO_3) were added as sources for DIP and DIN to create the desired DIP concentrations of 0.0, 0.2, 0.4, 0.8, 1.5, 3.0, and $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ and DIN concentration of $50 \mu\text{mol} \cdot \text{L}^{-1}$. The pH of the medium, after pasteurization, and DIN and DIP addition, was 8.1 ± 0.1 ($n = 14$) as measured with a pH-Meter (GHM-3511; Greisinger, Germany, Regenstauf).

Nutrient analysis. Dissolved inorganic nutrients (DIP and DIN) were measured with colorimetric analysis using a Technicon TRAACS 800 auto-analyzer (Seal Analytical, Germany, Norderstedt) in the NIOZ Texel nutrient laboratory. DIP was measured as ortho-phosphate (PO_4^{3-}) at 880 nm after the formation of molybdophosphate complexes (Murphy and Riley 1962). For DIN measurements (nitrate and nitrite), nitrate was first reduced to nitrite through a copperized cadmium coil and color intensity was measured at 550 nm after complexation with sulphonylamide and naphthylethylenediamine (Grasshoff 1983). Ammonium (NH_4^+) was measured at 630 nm after the formation of an indophenol blue complex with phenol and sodium hypochlorite at a pH of 10.5. Citrate was used as a buffer and complexant for calcium and magnesium at this pH (Koroleff 1972 and optimized by Helder and de Vries 1979). The low NH_4^+ -concentration ($0.022 \mu\text{mol} \cdot \text{L}^{-1}$) was not further considered, as no NH_4^+ was added in the experiments. The precision for all the measured channels within the automated nutrient analyzer was better than 0.25% (K. Bakker, pers. comm.).

DIP and DIN uptake dynamics. DIP and DIN uptake refers to the removal of these nutrients from the medium by *Saccharina latissima* and *Laminaria digitata*. Daily uptake rates (V) were derived from changes in the nutrient concentrations of the seawater medium each day, which were normalized for SA (cm^2) and time (day) using the following calculation:

$$V = (T_1 - T_2) \times \text{SA}^{-1} \cdot \text{t}^{-1}$$

with T_1 as the initial nutrient concentration, T_2 as the nutrient concentration before water exchange after 24 h, SA as SA (cm^2), and t as the incubation time (hours).

Two different uptake rates were classified over time: surge uptake (V_S) after starvation and maintenance uptake with filled nutrient pools (V_M). V_S was calculated from uptake rates under conditions of nonlimiting nutrient concentration using the following equation:

$$V_S = (V_2 - V_1) \times (d_2 - d_1)^{-1} = \Delta V \times \Delta d^{-1}$$

where V_1 and V_2 are daily uptake rates on days before a significant decline in uptake rate occurs and no significant variations in nutrient uptake follow. The difference operator between the 2 d is represented by d_1 and d_2 . V_M is calculated as the average uptake rate under nonlimiting nutrient concentration after a significant decrease has occurred and subsequent uptake rates show no significant variations.

Surface area analysis. Sporophytes of both species were individually spread flat on a white background, placed next to a ruler for scale, and covered with a transparent Plexiglas sheet to avoid folding of the frond. Photographs (using a Panasonic Lumix DMC-FT5) were taken on a weekly basis, enabling analysis of SA using the open source software

ImageJ (ImageJ; U.S. National Institutes of Health, Bethesda, MD, USA). Photographs were converted into grayscale (type 8-bit) and transformed into a binary image before SA analysis. The obtained SA represents one side of the frond. Differences in SA over time were used as indices of growth, with relative growth rates (μ) calculated according to Kain (1987) as follows:

$$\mu = (\ln \text{SA}_1 - \ln \text{SA}_2) \times \text{t}^{-1}$$

where SA_1 represents the initial surface area, and SA_2 represents the final surface area after incubation time t .

Experimental approach 2. In experimental approach 2, young sporophytes of *Saccharina latissima* and *Laminaria digitata* were placed inside 20 L bottles filled with 15 L DIP and DIN enriched seawater medium (DIP: $3 \mu\text{mol} \cdot \text{L}^{-1}$, DIN: $50 \mu\text{mol} \cdot \text{L}^{-1}$) for a 21-d adaptation phase under laboratory conditions. The nutrient-enriched seawater was renewed every other day to ensure saturated storage pigments after the adaptation phase. In experimental approach 2, individual sporophytes of *S. latissima* ($n = 20$) and *L. digitata* ($n = 20$) were transferred from the 20 L bottles into 500 mL glass jars filled with 200 mL seawater medium, which were either DIP and DIN enriched (DIP: $3 \mu\text{mol} \cdot \text{L}^{-1}$, DIN: $50 \mu\text{mol} \cdot \text{L}^{-1}$, $n = 5$), DIP depleted and DIN enriched (DIP: $0 \mu\text{mol} \cdot \text{L}^{-1}$, DIN: $50 \mu\text{mol} \cdot \text{L}^{-1}$, $n = 5$), DIP-enriched and DIN depleted (DIP: $3 \mu\text{mol} \cdot \text{L}^{-1}$, DIN: $0 \mu\text{mol} \cdot \text{L}^{-1}$, $n = 5$), or DIP and DIN depleted ($n = 5$). The seawater media were refreshed on a daily basis throughout the experiment. Before refreshment of the seawater medium, fluorescence measurements (F_v/F_m) were conducted every other day (see section Fluorescence measurements), and after daily refreshment of the seawater medium, all jars were placed on a rotating table (100 rpm) to provide a moderate water movement, whereas a random distribution of the jars minimized differences in light availability.

Fluorescence measurements. Fluorescence measurements to determine photosynthetic efficiency (F_v/F_m , F_v refers to variable fluorescence and F_m refers to maximum fluorescence) were conducted every other day over a period of 66 d for *Saccharina latissima* ($n = 5$) and 54 d for *Laminaria digitata* ($n = 5$) in all four treatments of experiment II. Sporophytes were dark-adapted for 20 min before photosynthetic efficiency was measured using a pulse-amplitude modulated fluorimeter (JUNIOR-PAM; Walz, Effeltrich, Germany; settings: measuring light intensity = 10, pulse width = 0.8 s, gain = 2) attached to a laptop. These measurements were carried out under minimum light conditions (laptop screen as the only light source) in a temperature-controlled room set to 12°C around the same daytime. Each sporophyte was measured twice at different locations on the frond in an interval of 40 s.

Internal storage capacity. The ISC for DIP and DIN were derived from the response of seaweed F_v/F_m when cultured in DIP- and DIN-depleted seawater. All, under the premise that internal storages for DIP and DIN had been filled during adaptation phase and the seaweed was not nutrient starved at the start of the experiment. Either DIN or DIP concentrations that were retrieved as nonlimiting in previous experimental set-up I were pulsed for potentially optimal conditions. A control was installed, adding both nutrients, DIP and DIN concentrations. A significant decrease in F_v/F_m under limitation/depletion conditions was postulated to reflect a stress reaction by seaweeds to internal DIP and/or DIN depletion, as parameters, like temperature, light, and hydrodynamics were fully controlled. As V_M is considered equal to the rate of assimilation (Taylor and Rees 1999, Barr et al. 2004), the ISC was calculated as follows:

$$\text{ISC} = \Delta d \cdot V_M$$

where Δd represents the duration (days) with initially filled internal nutrient storages under depletion conditions, before a significant decrease in F_v/F_m occurred, and V_M represents the daily maintenance or metabolic uptake rate.

Statistics. Data of both experimental approaches were tested for normality with the Kolmogorov–Smirnov test for cumulative probability distribution. A two-sided repeated measures ANOVA was applied to test for significant differences in growth, nutrient uptake rates, and F_v/F_m within and between treatments with different nutrient concentrations.

RESULTS

Experimental approach 1. Surface area analysis: The increase in SA, as a measure of growth of *Saccharina latissima* and *Laminaria digitata* sporophytes displayed significant differences between DIP treatments over time (respectively ANOVA, $F_{3,6} = 2.24$, $P = 0.042$; $F_{5,6} = 9.47$, $P < 0.001$). The highest growth rates for *S. latissima* were found in low to intermediate DIP treatments receiving nominal concentrations of $3.0 \mu\text{mol} \cdot \text{L}^{-1}$ or less, which were not significantly different in growth from each other (ANOVA, $F_{3,5} = 2.28$, $P = 0.545$). Mean SA increased by the factor 1.84 ± 0.14 in 23 d ($n = 42$; Fig. 2), representing a growth rate of 4% per day. *Saccharina latissima* cultured in high nominal DIP concentrations of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ exhibited significantly lower growth compared with sporophytes in other treatments (ANOVA, $F_{1,6} = 4.04$, $P = 0.004$). Mean SA increased by the factor 1.19 ± 0.21 in 9 d, before growth stagnated after 15 d and a negative growth with signs of texture loss and disintegration of the sporophytes was observed on day 23, the final measurement of SA ($n = 7$; Fig. 2).

Laminaria digitata showed the highest growth rates when exposed to intermediate nominal DIP concentrations of $1.5 \mu\text{mol} \cdot \text{L}^{-1}$ and $3.0 \mu\text{mol} \cdot \text{L}^{-1}$, and there were no significant differences in relative increase in SA among these treatments (ANOVA, $F_{1,6} = 0.46$, $P = 0.502$). Mean SA increased by the factor 2.37 ± 0.08 in 35 d ($n = 14$; Fig. 2), exhibiting a growth rate similar to *Saccharina latissima* in low to intermediate DIP treatments. Sporophytes cultivated under low nominal DIP conditions of $0.8 \mu\text{mol} \cdot \text{L}^{-1}$ or less, showed a significantly smaller increase in SA (ANOVA, $F_{3,6} = 3.39$, $P < 0.001$), which was comparable to *L. digitata* exposed to high nominal DIP concentration of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ (ANOVA, $F_{1,6} = 11.1$, $P = 0.001$). The relative increase in SA of sporophytes in these treatments increased by the factor 1.86 ± 0.05 , respectively, 1.81 ± 0.05 , in 35 d ($n = 28$, respectively, $n = 7$; Fig. 2), which translates to a growth rate of 2% per day.

DIP-uptake dynamics. Sporophytes of *Saccharina latissima* exposed to very low nominal DIP concentration of $0.2 \mu\text{mol} \cdot \text{L}^{-1}$, $0.4 \mu\text{mol} \cdot \text{L}^{-1}$ and $0.8 \mu\text{mol} \cdot \text{L}^{-1}$ depleted all supplied DIP within the daily sampling period of 24 h throughout the experiment, which indicates nonsaturating DIP concentrations to the nutrient starved sporophytes (not depicted). When exposed to nominal DIP concentrations of $1.5 \mu\text{mol} \cdot \text{L}^{-1}$, all supplied DIP was depleted until day 9, after which uptake significantly decreased (ANOVA, $F_{1,6} = 6.37$, $P = 0.021$) and mean uptake rates leveled off from $0.30 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ to $0.22 \pm 0.01 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ until day 22 ($n = 7$; Fig. 3A) with no significant variations (ANOVA, $F_{1,12} = 1.38$, $P = 0.220$), indicating saturating DIP conditions. Similarly, but with uptake declining earlier, sporophytes grown in a nominal DIP concentration of $3.0 \mu\text{mol} \cdot \text{L}^{-1}$ depleted all daily supplied DIP until

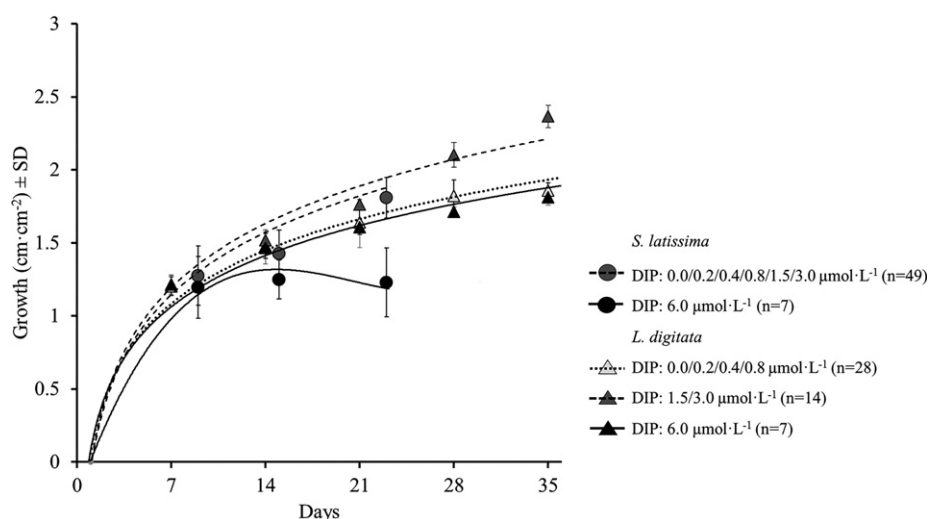


FIG. 2. Mean growth \pm SD ($\text{cm} \cdot \text{cm}^{-2}$) of young *Saccharina latissima* and *Laminaria digitata* cultivated in different DIP concentration ($0\text{--}6 \mu\text{mol} \cdot \text{L}^{-1}$, $n = 7$) and saturating DIN concentration ($50 \mu\text{mol} \cdot \text{L}^{-1}$) in a “pulse-and-chase” assay over 5 weeks. Data are depicted according to significant differences in increase in surface area (growth) of the sporophytes in different DIP concentrations.

day 4, followed by a significant decline of mean uptake rates (ANOVA, $F_{1,6} = 5.91$, $P = 0.007$) from $0.80 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ to $0.40 \pm 0.04 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ on day 7 ($n = 7$; Fig. 3B), after which no significant variations in DIP uptake rates were found (ANOVA, $F_{1,14} = 1.29$, $P = 0.226$). *Saccharina latissima* exposed to a high nominal DIP concentration of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ showed highly significant variations in DIP uptake within treatments (ANOVA, $F_{1,6} = 7.31$, $P < 0.001$) and over time (ANOVA, $F_{1,21} = 5.79$, $P < 0.001$). Sporophytes depleted all supplied DIP on days 1 and 2 with a mean uptake rate of $1.66 \pm 0.10 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 7$), which was followed by a daily decline and final collapse of mean DIP uptake rates to $0.05 \pm 1.28 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 7$) on days 21 and 22 (Fig. 3C). At this point, five of seven young sporophytes had lost their texture and started to disintegrate.

Based on DIP uptake rates of *Saccharina latissima* under saturated states in nominal DIP concentrations

of $1.5 \mu\text{mol} \cdot \text{L}^{-1}$ and $3.0 \mu\text{mol} \cdot \text{L}^{-1}$, the calculated $V_{\text{M-DIP}}$ was $0.30 \pm 0.09 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 14$).

V_{S} for DIP was calculated to be $0.80 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (average \pm SD, $n = 7$), which was based on DIP uptake rates of sporophytes exposed to a nominal concentration of $3.0 \mu\text{mol} \cdot \text{L}^{-1}$ on days 1 to 4. A maximum surge uptake rate was calculated to be $1.66 \pm 0.10 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (average \pm SD, $n = 7$), based on DIP uptake rates of the young sporophytes exposed to a nominal DIP concentration of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ on day 1. Sporophytes in this treatment disintegrated within 3 weeks and high uptake rates were referred to as a stress reaction to the unusually high DIP concentrations.

Sporophytes of *Laminaria digitata* cultured in nominal DIP concentrations of $0.2 \mu\text{mol} \cdot \text{L}^{-1}$, $0.4 \mu\text{mol} \cdot \text{L}^{-1}$, $0.8 \mu\text{mol} \cdot \text{L}^{-1}$, $1.5 \mu\text{mol} \cdot \text{L}^{-1}$, and $3.0 \mu\text{mol} \cdot \text{L}^{-1}$ depleted all of the supplied DIP within the 24-h sampling period throughout the experiment, which indicates nonsaturating DIP concentrations (depicted for

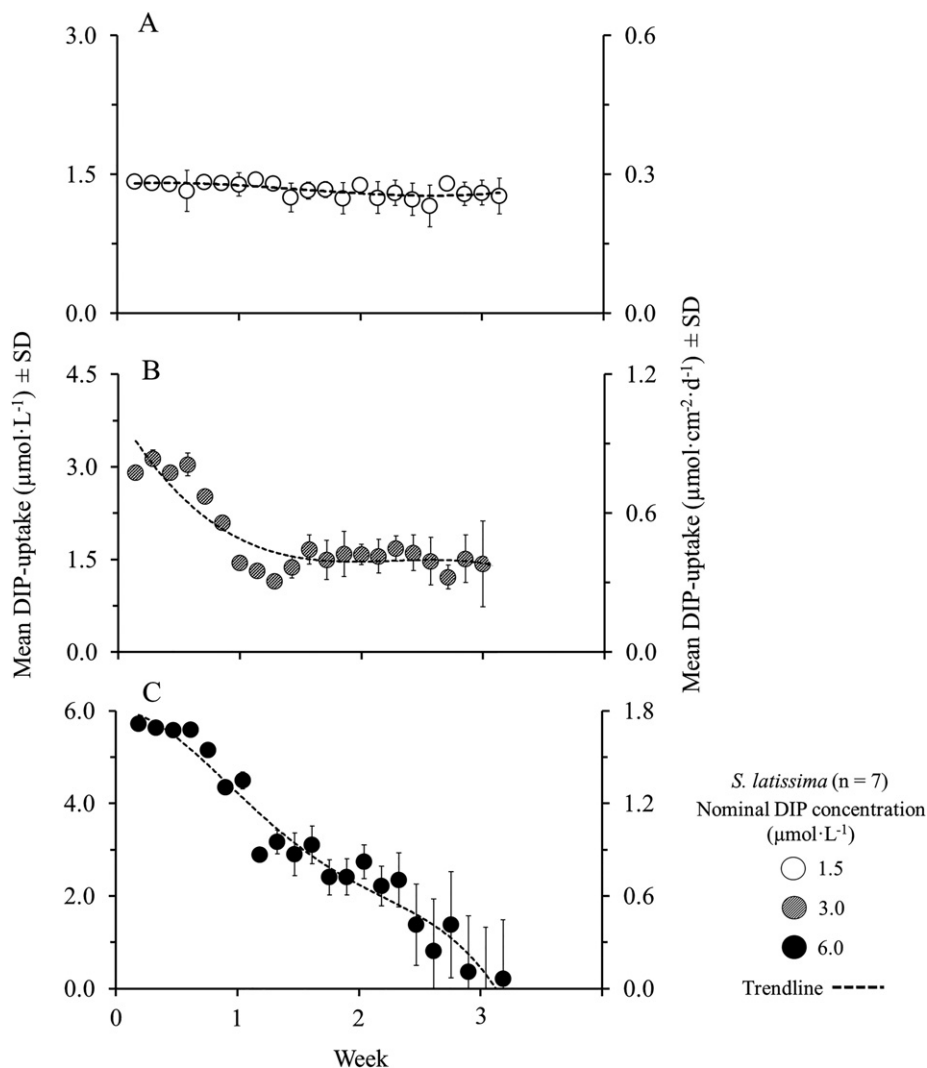


FIG. 3. Mean DIP uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Saccharina latissima* ($n = 7$) in saturating nominal DIP concentrations of (A) $1.5 \mu\text{mol} \cdot \text{L}^{-1}$, (B) $3.0 \mu\text{mol} \cdot \text{L}^{-1}$, and (C) $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIP uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a "pulse-and-chase" assay over 3 weeks.

$3.0 \mu\text{mol} \cdot \text{L}^{-1}$; Fig. 4A). In contrast, mean DIP uptake of *Laminaria digitata* cultured in a high nominal concentration of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ did not lead to depletion throughout the experiment, thus indicating a saturating concentration. Mean DIP uptake rates varied around $0.37 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ between day 1 and day 10 before a significant decrease occurred (ANOVA, $F_{1,11} = 8.50$, $P = 0.013$). Within day 11 and 21, mean uptake rates stabilized at $0.24 \pm 0.04 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 7$; Fig. 4B). The $V_M\text{-DIP}$ of *L. digitata* was calculated to be $0.22 \pm 0.01 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 14$), whereas $V_S\text{-DIP}$ was determined to be approximately twice as high as $V_M\text{-DIP}$ at $0.37 \pm 0.03 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ (average \pm SD, $n = 7$).

DIN-uptake dynamics: *Saccharina latissima* showed no significant differences in daily DIN uptake rates among different DIP treatments (ANOVA, $F_{6,6} = 1.71$, $P = 0.116$), but displayed a highly significant difference in uptake over time (ANOVA, $F_{6,21} = 5.35$, $P < 0.000$). No correlation between DIN and DIP uptake ($R = 0.415$) was found. The mean DIN uptake oscillated downwards from a high of $11.26 \pm 0.56 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 49$) on day 1, which represents the $V_S\text{-DIN}$ of *S. latissima*, to $5.46 \pm 0.77 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 49$) by day 14. After these 2 weeks, the DIN uptake stabilized around $4.07 \pm 0.82 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ without any significant variation (ANOVA, $F_{6,7} = 1.94$, $P = 0.097$) until the end of the experiment on day 22 (Fig. 5). A $V_M\text{-DIN}$ of $3.94 \pm 0.67 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ was conclusively calculated, which is approximately three times lower than $V_S\text{-DIN}$.

Laminaria digitata showed no significant differences in DIN uptake rates among different DIP treatments (ANOVA, $F_{6,6} = 1.21$, $P = 0.306$), but exhibited a highly significant difference in DIN uptake over time (ANOVA, $F_{6,20} = 28.46$, $P < 0.001$). Similar to *Saccharina latissima*, no correlation between DIN and DIP uptake ($r = 0.229$) was found. DIN uptake rates showed no significant variations within day 1 and 8 (ANOVA, $F_{6,7} = 0.27$, $P = 0.897$) with a mean uptake of $3.72 \pm 0.56 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 49$; Fig. 6). In correspondence, a $V_S\text{-DIN}$ of $3.92 \pm 0.08 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ ($n = 49$) was calculated. A significant decrease in uptake rates was observed within day 9 and 14 (ANOVA, $F_{5,6} = 5.44$, $P = 0.001$). After this, DIN uptake stabilized without significant variations (ANOVA, $F_{4,6} = 0.70$, $P = 0.590$) at $1.81 \pm 0.38 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ between day 16 and 21 (Fig. 6), which also represents $V_M\text{-DIN}$ for *L. digitata*.

Experimental approach 2. Based on the results on DIP and DIN uptake kinetics for *Saccharina latissima* and *Laminaria digitata* and in regard to saturating concentrations in experimental approach 1, nominal concentrations of $3.0 \mu\text{mol} \cdot \text{L}^{-1}$ DIP and $50 \mu\text{mol} \cdot \text{L}^{-1}$ DIN were chosen in experimental approach 2 for the control group ($n = 5$) to ensure nonlimiting nutrient availability throughout the experiment without inducing nutritional stress.

Fluorescence measurements: The mean photosynthetic efficiency (F_v/F_m) of *Saccharina latissima* showed significant variations between treatments (ANOVA, $F_{3,4} = 17.78$, $P < 0.001$) and over time

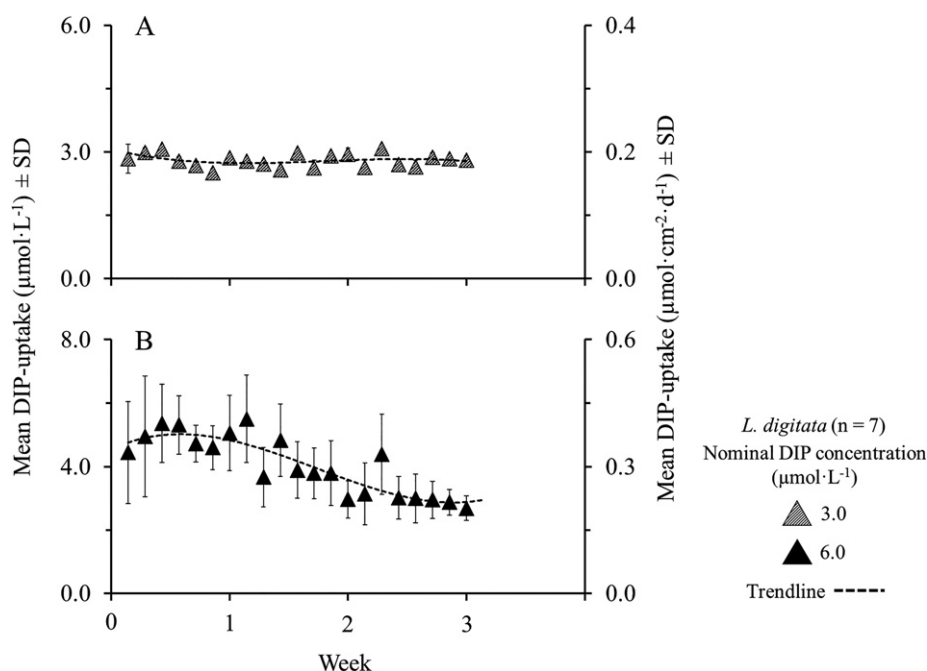


FIG. 4. Mean DIP uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Laminaria digitata* ($n = 7$) in (A) un-saturating nominal DIP concentration of $3.0 \mu\text{mol} \cdot \text{L}^{-1}$ and (B) saturating nominal DIP concentration of $6.0 \mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIP uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a "pulse-and-chase" assay over 3 weeks.

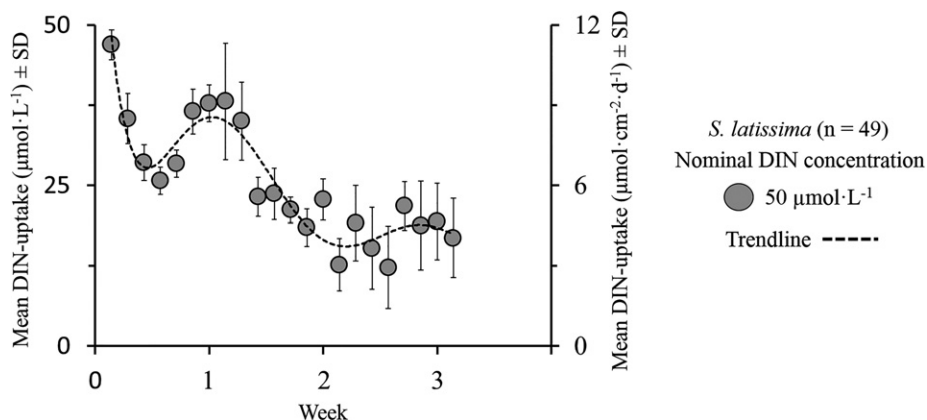


FIG. 5. Mean DIN uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Saccharina latissima* ($n = 49$) cultivated in nominal DIN concentration of $50 \mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIN uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a “pulse-and-chase” assay over 3 weeks.

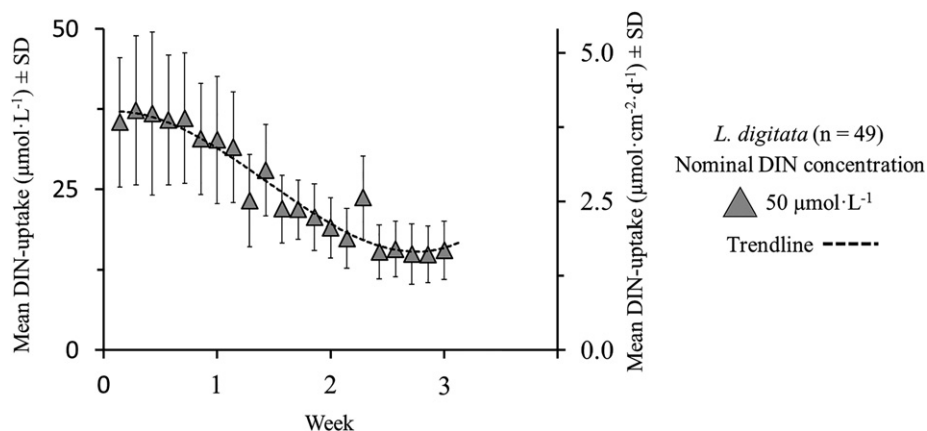


FIG. 6. Mean DIN uptake ($\mu\text{mol} \cdot \text{L}^{-1}$) \pm SD of young *Laminaria digitata* ($n = 49$) cultivated in nominal DIN concentration of $50 \mu\text{mol} \cdot \text{L}^{-1}$ and corresponding standardized daily DIN uptake ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$) in a “pulse-and-chase” assay over 3 weeks.

(ANOVA, $F_{3,33} = 5.09$, $P < 0.001$). The control group exposed to treatments with DIP and DIN additions of $3 \mu\text{mol} \cdot \text{L}^{-1}$ and $50 \mu\text{mol} \cdot \text{L}^{-1}$, respectively, *Saccharina latissima* expressed no significant differences in mean F_v/F_m (ANOVA, $F_{1,4} = 0.18$, $P = 0.686$), but displayed moderate fluctuations around a F_v/F_m of 0.78 ± 0.04 ($n = 5$) over 10 weeks (Fig. 7A). A comparable performance in F_v/F_m was observed in treatments under DIP-depleted conditions, where no significant difference in photosynthetic efficiency was found (ANOVA, $F_{1,4} = 3.58$, $P = 0.095$) and mean F_v/F_m stayed around 0.77 ± 0.04 throughout the experiment ($n = 5$; Fig. 7B). When exposed to DIN-depleted conditions, however, mean F_v/F_m significantly decreased (ANOVA, $F_{4,23} = 2.04$, $P = 0.007$) from 0.78 ± 0.04 to 0.70 ± 0.08 after 6 weeks ($n = 5$; Fig. 7C) with no significant variations thereafter (ANOVA, $F_{4,10} = 0.17$, $P = 0.998$). Similarly, mean F_v/F_m of *S. latissima* sporophytes exposed to total DIP and DIN depletion displayed no significant

variations until week 7 (ANOVA, $F_{4,22} = 1.35$, $P = 0.177$), followed by a significant decrease (ANOVA, $F_{1,4} = 2.37$, $P = 0.002$) from 0.77 ± 0.03 to 0.65 ± 0.16 during week 10 ($n = 5$; Fig. 7D).

The photosynthetic efficiency of *Laminaria digitata* also exhibited significant differences between different treatments (ANOVA, $F_{3,4} = 11.79$, $P < 0.001$) and over time (ANOVA, $F_{4,22} = 5.26$, $P > 0.001$). Sporophytes of the control group exposed to a DIP and DIN concentration of $3 \mu\text{mol} \cdot \text{L}^{-1}$ and $50 \mu\text{mol} \cdot \text{L}^{-1}$, respectively, showed no significant variations in F_v/F_m over time (ANOVA, $F_{4,22} = 0.77$, $P = 0.406$) and the mean photosynthetic efficiency was 0.74 ± 0.06 throughout the experiment ($n = 5$; Fig. 7E). However, photosynthetic efficiency was more sensitive to DIP and DIN depletion than in *Saccharina latissima*. When exposed to DIP-depleted, DIN-depleted, and both DIP and DIN-depleted seawater medium, the mean F_v/F_m significantly decreased after 6 weeks (ANOVA, DIP-depleted: $F_{4,22} = 2.41$, $P = 0.025$; DIN-depleted: $F_{4,22} = 8.51$, $P = 0.043$; DIP/DIN-depleted:

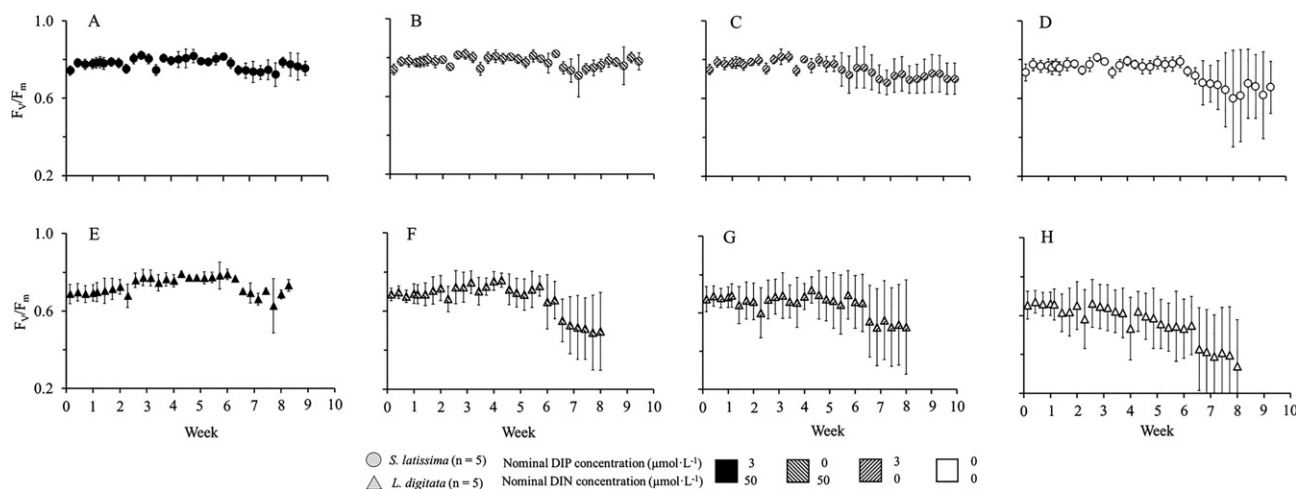


FIG. 7. Photosynthetic efficiency F_v/F_m of *Saccharina latissima* (A–D, $n = 5$) and *Laminaria digitata* (E–H, $n = 5$) cultivated in DIP/DIN-enriched (A and E), DIP-depleted (B and F), DIN-depleted (C and G), and DIP/DIN-depleted (D and H) seawater medium in a “pulse-and-chase” assay over 10 weeks, respectively, 8 weeks.

$F_{4,22} = 2.40$, $P < 0.001$). Under DIP depletion, the mean photosynthetic efficiency dropped from 0.73 ± 0.05 to 0.50 ± 0.20 during week 8 ($n = 5$; Fig. 7F), while mean F_v/F_m under DIN depletion decreased comparably from 0.67 ± 0.10 to 0.53 ± 0.25 during week 8 ($n = 5$; Fig. 7G). *Laminaria digitata* sporophytes under DIP and DIN depletion displayed a moderate decrease in mean F_v/F_m from 0.67 ± 0.07 to 0.55 ± 0.15 between week 3 and 6, after which the mean photosynthetic efficiency significantly dropped to 0.34 ± 0.24 during week 8 ($n = 5$; Fig. 7H).

Internal storage capacity. Using the fluorescence measurements and duration time, before a significant decrease in F_v/F_m in different treatments occurred and the daily DIP and DIN uptake rates under V_M , we calculated an ISC of $10 \mu\text{mol} \cdot \text{cm}^{-2}$ ($n = 7$) for DIP and $80 \mu\text{mol} \cdot \text{cm}^{-2}$ ($n = 49$) for DIN in *Laminaria digitata*. An ISC-DIN of $160 \mu\text{mol} \cdot \text{cm}^{-2}$ ($n = 49$) was calculated for *Saccharina latissima*, while ISC-DIP could not be calculated from the experimental data collected on this species as no indication of phosphorus nutritional stress was exhibited and no significant decrease in mean F_v/F_m was observed over 66 d (Fig. 7B). However, based on the DIP requirements according to V_M over 66 d and consistent with a DIP:DIN uptake ratio of 1:6 under steady-state conditions, we estimated an ISC-DIP of $27 \mu\text{mol} \cdot \text{cm}^{-2}$ ($n = 14$) for *S. latissima*.

DISCUSSION

The growth, productivity, and geographical distribution of seaweeds are controlled by environmental factors, such as temperature, irradiance, water movement, and nutrient availability. Seasonal fluctuations in nutrient availabilities can also reflect differences in the seasonal growth patterns of seaweeds (Gagne et al. 1982, Zimmerman and Kremer 1986), as for *Saccharina latissima* and *Laminaria digitata* (Conolly

and Drew 1985). This study adds to the physiological understanding of dissolved inorganic P and N uptake in *S. latissima* and *L. digitata*, which in turn enables estimation of ecological effects on nutrient availability.

Uptake kinetics are usually expressed as functions of either fresh weight (FW), dry weight (DW) or surface area to volume (SA:Vol), which makes it difficult to compare data. Furthermore, uptake kinetics expressed as a function of DW necessitates destructive sampling through harvesting living biomass. Standardized determination by FW is even more problematic as small variations in the amount of water attached to the living (and growing) seaweed can lead to large differences in its measured weight, not only between different samples and over time, but also amongst different experimenters. As seaweeds take up nutrients throughout their whole frond, the SA represents a reasonable function to determine uptake kinetics. Standardization of uptake kinetics by SA would allow for intra- and interspecific comparisons over time, for example, with observations on the green seaweed *Ulva lactuca* by Lubsch and Timmermans (2018). Moreover, phenotypic plasticity of seaweed strongly depends on predominant hydrodynamics of the site (Gerard 1987, Demes et al. 2011), but can also be affected by biotic stress (Molis et al. 2015), which can make comparison of functions of SA:Vol troublesome. Comparisons between uptake kinetics, such as V_S , V_M , and ISC, of different seaweed species would allow for general insights into seaweed survival and competition in natural environments. It is also an important aspect in scaling up operations to levels of commercial viability, as it enables to estimate the carrying capacity of a cultivation site in regard to nutrient availability and nutrient demand of cultivated species, as well as it allows to adjust duration and quantity of potential nutrient additions

according to size (seaweed $SA \cdot m^{-2}$) and growth of the operation.

Our results on growth rates for both, *Saccharina latissima* and *Laminaria digitata*, with only a slightly sub-optimal increase in SA observed under nonsaturating external DIP conditions within the first 28 d of the first experimental approach, suggests that previously filled internal phosphate storages were utilized during the experiments and were able to compensate for external DIP deficiency (after Probyn and Chapman 1982, Pederson and Borum 1996). This is supported by the reduced, but continuing growth of both species when exposed to DIP-depleted seawater, which clearly indicates that internal phosphate storages had not been depleted after 2 weeks of starvation during the adaptation phase. The mean growth rates of $4\% \cdot d^{-1}$ under optimal DIP conditions for *S. latissima* and *L. digitata* are within the reported growth rates for both species from the North Sea area (*S. latissima*, Nielsen et al. 2014, Boderskov et al. 2015; *L. digitata*, Gomez and Lüning 2001). The reduced growth of *L. digitata* exposed to DIP concentrations of $6.0 \mu mol \cdot L^{-1}$, which are above the optimal levels, has also been observed for *Ulva lactuca* by Waite and Mitchell (1972) and Steffensen (1976). These studies showed that phosphate concentrations above $4.76 \mu mol \cdot L^{-1}$ had negative effects on growth rates for the green seaweed. Similarly, the daily pulsing of high DIP concentrations had a fatal effect on *S. latissima*.

The texture loss and disintegration of juvenile *Saccharina latissima* sporophytes exposed to nominal DIP concentration of $6.0 \mu mol \cdot L^{-1}$ within 3 weeks of exposure could have been caused by epiphytic bacteria. In many cases the level of bacterial populations found on seaweed surfaces depend on the species, thallus section, and season (Armstrong et al. 2000, Bengtsson et al. 2010). One cause of a seasonal shift in marine and epiphytic bacterial communities may be a change in external conditions or physical and chemical parameters, such as nutrient stoichiometry and availability. However, studies have demonstrated the ability of certain marine bacteria to degrade various seaweed polymers (Goecke et al. 2010), thus leading to fouling and disintegration of the seaweed. However, *S. latissima* sporophytes only started to disintegrate after 3 weeks of exposure to nominal DIP concentration of $6.0 \mu mol \cdot L^{-1}$, and the daily uptake rates within the first week showed the ability of juvenile sporophytes to manage pulses of high DIP concentration for a short time. This ability could also be altered when stress reactions to high external nutrient concentration are initiated (e.g., Fourcroy 1999, Jiang and Yu-Feng 2008), allowing for mobilization and uptake of sufficient DIP to provide temporary relief.

Two different phases of transient responses to nutrient pulses, an initial surge uptake rate (V_S) after starvation and a maintenance or steady-state uptake rate

(V_M), which is considered equal to the rate of assimilation (Taylor and Rees 1999, Barr et al. 2004) were clearly seen for DIP and DIN-uptake in *Saccharina latissima* and *Laminaria digitata* in our first experimental approach. Depending on total DIP availability, V_S -DIP in *S. latissima* was maintained until the internal storages had been filled and uptake rates gradually decreased to V_M -DIP levels. This is supported by a significant decrease in DIP uptake found in the treatments with saturating nominal DIP concentrations of $1.5 \mu mol \cdot L^{-1}$ and $3.0 \mu mol \cdot L^{-1}$ on day 9 and day 4, respectively. Similar uptake characteristics were found for *L. digitata* exposed to a nominal DIP concentration of $6.0 \mu mol \cdot L^{-1}$; thus, similarly starved *L. digitata* exposed to a nominal DIP concentration twice as high may lead to a shift in uptake rates from V_S to V_M in approximately half the time. This time-shifted phenomenon has also been described for DIP uptake in the green seaweed *Ulva lactuca* (Lubsch and Timmermans 2018) and corroborates evidence that the filling of internal nutrient pools is uncoupled from growth (Conway et al. 1976, Chapman et al. 1978). The high DIP uptake rates of *S. latissima* exposed to nominal concentrations of $6.0 \mu mol \cdot L^{-1}$ within the first 2 d, although referred to as a stress reaction allowing for temporary relief, shows the ability of *S. latissima* to "handle" high DIP concentrations for a short time indicated by a stagnation of growth after 9 d, and in turn the calculated V_S -DIP observed from sporophytes in $3.0 \mu mol \cdot L^{-1}$ within 4 d might have been underestimated. It should be mentioned that the daily offered nominal concentration of $6.0 \mu mol \cdot L^{-1}$ would correspond to an initial daily DIP availability of approximately $1.6 \mu mol \cdot cm^{-2} \cdot d^{-1}$ for *S. latissima* and $0.4 \mu mol \cdot cm^{-2} \cdot d^{-1}$ for *L. digitata*, depending on the SA of the sporophytes. Therefore, the nominal concentration does not represent a fully comparable measure between these species.

DIN uptake rates of *Saccharina latissima* and *Laminaria digitata* under fully saturating DIN conditions followed the same response as DIP uptake rates under saturating DIP conditions. V_M -DIN was attained when internal DIN pools had been filled. In regard to the filling of internal DIN pools, *S. latissima* showed a V_S three times higher than its V_M for DIN, as well as V_S for DIN in *L. digitata*. These values are comparable to the V_S and V_M for DIN, respectively, $12.5 \pm 5.2 \mu mol \cdot cm^{-2} \cdot d^{-1}$ and $2.3 \pm 0.9 \mu mol \cdot cm^{-2} \cdot d^{-1}$, found in the green seaweed *U. lactuca* (Table 1; Lubsch and Timmermans 2018). Nutrient uptake rates can also vary according to the seaweeds age, as uptake of nitrate in first-year plants of *Laminaria groenlandica* Rosenvinge was three times higher than in second- and in third-year plants (Harrison et al. 1986).

The oscillatory decrease in DIN uptake in *Saccharina latissima* during V_S suggests that DIN uptake was limited by internal aspects, such as the physical transfer of nutrients to inner tissue and/or enzymatic activity by feedback-controlled processes

at the molecular level. Evidence shows that cellular processes are intrinsically rhythmic and follow a circadian metabolic timekeeping. Although the molecular basis of circadian rhythms in seaweed is poorly understood, many circadian rhythms have been described for microalgae (Wijnen and Young 2006). For example, the green single-cell alga *Chlamydomonas reinhardtii* shows maximal daily uptake of DIN at dawn and maximal nitrate reductase activity around midday (Pajuelo et al. 1995). This rhythmic expression aids in the synchronization of mutually coupled dynamical systems (Prokhorov and Nijmeijer 1998). Furthermore, it has been demonstrated that species diversity could be enhanced by different temporal nutrient uptake pattern in micro-algae and even under limitation conditions a coexistence was possible (Ahn et al. 2002).

Survival and growth of perennials also depends on the duration of internal nutrient storages to overcome seasonal minima in nutrient availability. In experimental approach 2, the photosynthetic efficiency (F_v/F_m) was measured as an indication to nutrient stress. F_v/F_m of various seaweeds has often been applied to indicate stress resulting from desiccation (Varela et al. 2006, Schagerl and Möstl 2011, Flores-Molina et al. 2014), photo-period (Magnusson 1997) or light-intensity (Hanelt et al. 1997, Gevaert et al. 2002). The use of F_v/F_m as an indication of nutrient-related stress in the marine sector has been more common in microalgae (Kromkamp and Peene 1999) and corals (Wiedenmann et al. 2012). F_v/F_m values in the range of 0.79–0.84 are considered optimal for many plants, whereas values significantly below are considered to indicate stress (Kitajima and Butler 1975, Maxwell and Johnson 2000). Accordingly, *Saccharina latissima* and *Laminaria digitata* first indicated nutritional stress by a significant decrease in F_v/F_m after 9, respectively, 7 weeks of exposure to DIP- and DIN-depleted seawater. This decrease in F_v/F_m can be inferred to indicate depletion of internal storage pools of DIP and/or DIN, as abiotic parameters like light, temperature, and hydrodynamics were kept constant during the pulse-and-chase approach and in relation to the control.

The inferred ISC for DIP and DIN in *Saccharina latissima* and *Laminaria digitata*, derived

from uptake kinetics (experimental approach 1) and the observed photosynthetic efficiency F_v/F_m (experimental approach 2) are realistic for perennial seaweeds like *S. latissima* and *L. digitata*, which are considered K-strategists that seasonally store reserves. The reserve ratio of the ISC for DIN and DIP of 6:1 in *S. latissima* and 8:1 in *L. digitata*, compared to V_M , the rate of assimilation with DIN and DIP uptake ratios of approximately 13:1 for *S. latissima* and 8:1 for *L. digitata*, suggests that *S. latissima* is twice as likely to fall under N limitation than P limitation, whereas in *L. digitata* the storage ratio is equivalent to V_M . A pattern that was reflected by our results on F_v/F_m , which showed a decrease in photosynthetic efficiency to DIN and/or DIP depletion conditions at the same time in *L. digitata*, whereas *S. latissima* exhibited a decrease in F_v/F_m to DIN depletion, but none to DIP depletion, after 6 weeks of exposure. The high demand for DIN (and DIP) in *S. latissima* was also reflected by its high uptake rates under V_S (Table 1), which is comparable to the V_S for DIN and DIP in the opportunistic green seaweed *U. lactuca* (Lubsch and Timmermans 2018), which is considered a promising seaweed for biofiltration purposes (Neori et al. 2003). Unlike *U. lactuca*, which flourishes at relatively high temperatures and light intensities, *S. latissima* can be regarded as a winter species, and this could allow for crop rotation in mariculture. Our data provide evidence that *S. latissima* is an effective candidate for bioremediation, for example, in close proximity to marine fish farms, potentially able to balance nutrient loads from fish cages, whereas a relatively fast growth provides valuable biomass at the same time (Handa et al. 2013, Reid et al. 2013, Freitas et al. 2015).

Based on our results on DIP and DIN uptake kinetics and calculated ISC, *Saccharina latissima* is predicted to outcompete *Laminaria digitata* in the struggle for nutrients, despite similar spatial and temporal distribution. As mentioned before, multiple environmental factors regulate geographical distribution, and there is no available information about sporophyte recruitment strategies and the intra- and interspecific competitiveness of gametophytes of *Saccharina latissima* and *Laminaria digitata*.

TABLE 1. Calculated dissolved inorganic phosphate (DIP) and dissolved inorganic nitrate (DIN) surge uptake rates (V_S), metabolic uptake rates (V_M), and internal storage capacity (ISC) of *Saccharina latissima*, *Laminaria digitata* and *Ulva lactuca*.

	DIP			DIN		
	$V_S, \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$	$V_M, \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$	ISC, $\mu\text{mol} \cdot \text{cm}^{-2}$	$V_S, \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$	$V_M, \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$	ISC, $\mu\text{mol} \cdot \text{cm}^{-2}$
<i>S. latissima</i>	0.80 ± 0.03	0.30 ± 0.09	27 ^a	11.26 ± 0.56	3.94 ± 0.67	160 ^a
n	7	14	14	49	49	49
<i>L. digitata</i>	0.38 ± 0.03	0.22 ± 0.01	10 ^a	3.92 ± 0.08	1.81 ± 0.38	80 ^a
n	7	14	7	49	49	49
<i>U. lactuca</i> ^b	0.66 ± 0.12	0.07 ± 0.04	0.7 ± 0.1	12.5 ± 5.2	2.3 ± 0.9	23 ± 7
n	3	6	9	24	24	24

^aApproximation based on V_M , DIP:DIN uptake ratio, and photosynthetic efficiency F_v/F_m over time.

^bData derived from Lubsch and Timmermans (2018).

Reed (1990) showed that intra- and interspecific competition was more intense when settlement of gametophytes of *Macrocystis pyrifera* and *Pterygophora californica* were at high densities, but not at low densities. On the other hand, no evidence of competition was found among gametophytes of *Nereocystis luetkeana* (Vadas 1972). Delaying development can also ameliorate the negative effects of intra- and interspecific competition among seaweed gametophytes (Carney and Edwards 2010).

Our standardized data add to the physiological understanding of *Saccharina latissima* and *Laminaria digitata* and can contribute to the development and modification of applications in a bio-based economy, such as in IMTA. Likewise, the obtained physiological data can help to identify potential locations for commercial cultivation and facilitates predicting yields of seaweed biomass in different locations under different environmental conditions using various models (Broch and Slagstad 2012, Van der Molen et al. 2018). These are important applications, as the interest in industrialization of seaweed culture has increased in Europe throughout the last decades (Holdt and Kraan 2011, Wijesinghe and Jeon 2012).

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